Proposal for the National Children's Study on Congenital Human Herpesvirus 6 Infection

I. Proposed Core Hypothesis:

Human herpesvirus 6 (HHV6) acquired congenitally results in neurodevelopmental deficits which may not be apparent at birth but rather are progressive, similar to the closely related herpesvirus, cytomegalovirus (CMV).

The risk of developing neurodevelopmental deficits, including sensorineural hearing loss, is partly dependent on the time of HHV6 transmission during pregnancy and virologic factors. Such factors include the HHV6 variant (or strain) the state of replication of the virus in the cord blood, the subsequent persistence in the infant, the sites of persistence, and the frequency of reactivation.

II. Work Groups:

- Primary Working Group: Infection, Immunity, and Vaccines.
- Potential Collaborating Work Groups: Pregnancy and Infant ,   Repository

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IV. Public Health Significance:

Congenital transmission of HHV6 occurs in 1% of births, the same rate as that of the closely related Human herpesvirus, CMV. Congenital CMV infection is initially asymptomatic in 90% of infants, but of these well-appearing infants approximately 15% will develop neurologic and/or hearing deficits over the first several years of life. Congenital CMV infection currently is the major identified cause of permanent hearing loss in children in the United States. Whether intrauterine HHV6 infection can result in hearing loss and neurodevlopmental delays is unknown. However, HHV6 has even greater tropism for the central nervous system than CMV[1, 2] and thus may cause an appreciable proportion of the over 5,000 infants born each year in the United States with moderate to profound bilateral hearing loss. Children with more subtle hearing deficits which are undetected at birth and those with unilateral hearing loss add a significant, but unestimated, number of children with permanent hearing loss.

Hearing loss acquired early in life can greatly affect a child's language, literacy, social, and emotional development. The ages from birth to 5 years are regarded as the critical period for development of language.[3, 4] Furthermore, it has been shown that normal hearing during the first 6 months of life is extremely important for normal acquisition of language and oral speech, and that infants identified with hearing impairment by 6 months of age have significantly better language outcomes than infants whose deficits are identified after 6 months of age.[5] However, the median age of identification for permanent moderate to severe hearing loss in infants in the United States has been 12 to 25 months.[6, 7]

In Rochester, NY, universal newborn hearing screening (as part of the New York State Universal Newborn Hearing Screen Demonstration Project) indicated a prevalence of newborns with severe to moderate hearing loss of 3.7 per 1,000.[8] Another 2 to 3 per 1,000 children are detected with permanent hearing loss over the next 2 to 3 years. Currently CMV is the most frequently identified cause of congenital hearing loss; however, in the Rochester, New York State

Demonstration Project over 90% of the infants had no identifiable cause of the permanent hearing loss.

Congenital Human herpesvirus infections also may result in other types of neurologic and developmental disabilities. The estimated prevalence of developmental disabilities ranges from 0.3–0.6/1,000 children for those with visual impairment to 75–150/1,000 children for those with learning disabilities, and 6–13% of all children are estimated to have significant behavioral disorders.[9] Currently the proportion of the disabilities related to or augmented by congenital herpesviruses infections is unknown. Most programs who care for such children are aimed at the diagnosis and therapeutic modalities available to manage children already severely compromised. Efforts at preventing such disabilities have been confounded by the lack of information of their causes, pathogenesis, and means of early recognition. Without such knowledge anticipatory programs for prevention cannot be instituted. Identification of the potential etiologies and the risk factors associated with congenitally acquired neurodevelopmental deficits could diminish the life-long sequelae currently incurred by such infants and decrease the considerable health care costs which occur from late diagnosis and management of disabilities which have already become irreversible.

V. Justification for a large, prospective, longitudinal study

Intrauterine transmission of HHV6 has only recently been identified. HHV6 DNA has been identified in 1% of cord bloods in one large study of approximately 6,000 cord bloods (Hall, 2002) and at rates of 1–1.6% in several smaller studies.[10-13] Thus, little is known about congenital HHV6 infection, and no studies have examined the importance of congenital HHV6 infection or the outcome of affected infants. Studies examining the subsequent course and outcome of infants with congenital HHV6 infection are potentially problematic as primary HHV6 infection is not only essentially a universal infection in the United States, but it occurs in about all children by 2 years of age.[2] Thus, children who acquire their HHV6 infection by the intrauterine route versus those who acquire it postnatally must be identified at birth by examination of the cord bloods and both cohorts prospectively followed to determine whether the outcome is related to the mode of acquisition. Furthermore, the neurodevelopmental abnormalities which may be associated with a congenital infection may not be apparent at birth, but progressive, requiring sequential prospective evaluations from birth. Cohort studies initiated later involving children with identified disabilities would not allow determination of the role of HHV6 infection in outcome as most children will already have evidence of HHV6 infection and the time of acquisition could not be determined retrospectively.

Investigation of the entire cohort of approximately 100,000 would be necessary because the frequency of congenitally transmitted HHV6 infection is low (about 1% of births) and the incidence of associated neurodevelopmental abnormalities is unknown. Furthermore, subgroup analysis would be necessary to determine the risk of developing specific types of neurologic and developmental deficits. In addition, congenital HHV6 infection may be a cofactor in the development of abnormalities associated with other causes, such as with other herpesviruses since *in vitro* and *in vivo* interactions of these viruses have been demonstrated.[14-16]

Since the frequency of deficits associated with HHV6 is unknown, the best estimation may be derived from the well studied observations of congenital CMV infection. As previously noted, HHV6 and CMV are closely related virologically and biologically. Of infants with congenital CMV infection who are asymptomatic at birth 10–15% will later develop neurodevelopmental abnormalities, approximately half of which are hearing loss. Although a similar incidence could be hypothesized for congenital HHV6 infection, it is even possible that a greater rate of neurodevelopmental abnormalities could occur with HHV6 since HHV6 infection appears to have a

neurotropism and persistence in the CNS greater than that described for CMV.[1, 2] The occurrences of congenital HHV6 and congenital CMV infections have been demonstrated to be 1%. Hence, from a sample size of 100,000, 1% or 1,000 would be expected to demonstrate congenital HHV6 infection. If 20% of these infants develop neurodevelopmental disabilities, 200 could be identified with abnormalities associated with congenital HHV6 infection. This cohort could be compared to the other 800 with congenital HHV6 infection who are without such abnormalities and compared to a third cohort of matched infants without congenital HHV6 infection who would be drawn from the 99,000 remaining infants who have no evidence of congenital HHV6 infection. These sample sizes would allow determination of whether there is a significant association of neurodevelopmental deficits in general with congenital HHV6 infection. The size of this sample also would be likely to detect significant associations with specific types of neurodevelopmental abnormalities and virologic factors, such as duration of persistence of HHV6 in peripheral blood mononuclear cells, variant, frequency of reactivation, and markers of humoral or cellular immunity. For example, sample sizes of 200 HHV6 cord blood positive (congenitally infected) infants and 200 cord blood negative (uninfected) infants (400 total) would provide 90% power to detect differences in means of 0.32 standard deviation units (a small effect size) between the groups, using a two-tailed t-test at the 5% level of significance. This calculation applies to detecting differences in antibody levels, viral load, and neurodevelopmental test scores at any time at or before 12–15 months of age.

VI. Scientific Merit

HHV6 and CMV are closely related herpesviruses, both virologically and biologically in their ability to persist, reactivate, and cause congenital infection in about 1% of births. A reasonable hypothesis is that HHV6, when acquired congenitally, also may result in a compromised neurologic or developmental outcome. As with congenital CMV infection, most HHV6 congenitally infected infants appear normal at birth, but with time, continuing HHV6 infection may lead to the progressive development of sensorineural hearing deficits and developmental delay over the first several years of life.[17-21] Neurologic abnormalities have long been associated with postnatally acquired primary HHV6 infection. Furthermore, HHV6 has been shown in some children to persist subsequently in the CSF and CNS.[1, 2] The course and outcome of children with congenitally acquired HHV6 infection as well as those with persistence of the HHV6 genome in the CSF and other body sites, have not been studied.

Compelling evidence, nevertheless, exists suggesting that congenital HHV6 infection may be associated with subsequent neurodevelopmental disabilities. HHV6 and CMV are two of the most closely related viruses within the herpesvirus family. Not only does the rate of intrauterine transmission of these two viruses appear to be the same, but the mechanism of placental transmission may be similar. Both viruses have been shown *in vitro* to infect human placental syncytiotrophoblast cells[22], and HHV6 DNA has been documented in the genital tract of up to 25% of pregnant and non-pregnant women, a rate similar to that reported for CMV DNA in maternal cervical secretions.[13, 23-30]

Adverse outcomes after intrauterine HHV6 infection are additionally suggested by case reports of infants who develop CNS disease and case reports identifying HHV6 DNA in aborted fetuses and placental tissue.[31-33]

The similarity of infants with congenital HHV6 infection to those with congenital CMV infection is further suggested by some common clinical and virologic findings. At birth most children with congenital HHV6 infection have been asymptomatic, as with CMV. Congenital HHV6 infection is not accompanied by concurrent viremia which is consistently seen with primary postnatal HHV6 infection, and congenital HHV6 infection as with congenital CMV infection is

subsequently associated with more frequent persistence of HHV6 DNA in various sites, such as the peripheral blood mononuclear cells and respiratory secretions. In our studies, levels of HHV6 IgG antibody present at birth appeared to correlate with protection against infection. The protective role of humoral antibody against infection has been clearly shown for CMV infection.[34-40] However, in contrast to congenital CMV infection, which occurs mainly from primary maternal infection, congenital HHV6 infection occurs in previously infected women since primary infection is universal during early childhood. Thus, congenital HHV6 infection presumably occurs from reactivation of previous maternal infection. Determination of the virologic factors, such as viral state of HHV6 when congenitally transmitted, therefore may be important as they may be indicators of the risk for subsequent neurodevelopmental disabilities.

The risk of neurologic sequelae in certain patients may be augmented if HHV6 infection occurs *in utero* during the critical periods of CNS development. Furthermore, HHV6's effect on the CNS over time may be to engender neurologic dysfunction in domains subserved by its areas of predilection, the temporal and frontal lobes, i.e. memory, attention, and behavior. Despite numerous case reports of CNS manifestations associated with HHV6 infection no study to date has assessed the subsequent developmental outcome or neurocognitive function associated with congenital HHV6 infection. Either or both may be affected adversely by a neurotropic viral infection early in life, and especially by one that persists in the immature developing CNS. A protective study would help delineate the frequency and type of neurodevlopmental abnormalities associated with congenital HHV6 infection, and the resulting burden in health care costs and morbidity. Host and virologic risk factors may be able to be defined which may lead to early detection and, more importantly, prevention of transmission of the maternal HHV6 infection to the infant.

VII. Potential for innovative research

Since essentially no information exists on this newly recognized mode of transmission for HHV6, intrauterine infection, multiple opportunities for productive and innovative research exist. First, potential studies of congenital HHV6 infection offer the unique opportunity to study a congenital viral infection acquired from reactivation of previous maternal infection, a means of congenital viral transmission that has been little recognized or studied. Second, many virologic characteristics of HHV6, such as persistence and variant, are of unknown clinical importance. For example, only variant B has been shown to cause primary infection. However, our recent studies have indicated primary infection with variant A may occur if infection is by the intrauterine route. Similarly, the immunomodulating effect of HHV6, which has been shown to occur *in vitro*, may be magnified in congenital HHV6 infection since it is acquired when the immune system is immature. This may allow a better understanding of the clinical importance of HHV6's potential for affecting the immune system throughout life. Additionally, an interaction of HHV6 with other herpesviruses, especially CMV, has been clearly shown in transplant patients, and reactivation of HHV6 has been associated with an increased severity of CMV disease.[14, 15, 41] Similarly, in the fetus and neonate coinfection of these two viruses may effect or augment the manifestations associated with either congenital infection and help explain the variation in manifestations and sequelae associated with CMV infection.

VIII. Feasibility

The cohort of 100,000 newborns would allow sufficient sample size for the detection of associated neurodevelopmental abnormalities at an acceptable level of significance as noted in section V. We have developed laboratory assays appropriate for determining the virologic factors which may be associated with the risk of intrauterine transmission and subsequent sequelae. Assays

for detecting HHV6 DNA in the cord blood to determine the frequency of congenital HHV6 infection are now well standardized[2] and we have recently developed assays for the determination of the viral state of HHV6 infection which include an RT-PCR for mRNA of a late structural gene (gp82–105), a marker of productive infection, and an RT-PCR for latency-associated transcripts (RT-PCR-LATS) associated with latent, non-productive infection.[42, 43] Additionally, we have developed a real time quantitative PCR for determination of the viral load of HHV6 which is variant specific.

Audiologic testing of neonates and of young children has been a focus of the research by our audiology group at the University of Rochester Medical Center, under the direction of Dr. Larry Dalzell.[8] Neonates are tested using otoacoustic emissions (OAE) and/or auditory brain stem responses which possesses a sensitivity of close to 100% for moderate or greater hearing loss in the newborn. Subsequent audiologic evaluations at 1 to 2 years of age are assessed by behavioral hearing testing, considered the reliable standard for audiologic evaluation in young children.[44] This technique includes visual re-enforcement audiometry which conditions a child as young as 6 to 12 months to associate a sound stimulus with a light toy re-enforcer, and "conditioned play" audiometry in which the child performs an act of play in response to sound.

New and innovative assessments of neurodevelopment, such as the visual expectation paradigm, as well as the Fagan Infantest, Bayley-II, and Delayed Response Tasks, have been successfully used at the University of Rochester in prospective studies of children with toxin exposure.[45, 46] These tests have allowed sensitive detection of neurodevelopmental deficits in very young infants and have been shown to be predictive of subsequent disability.[45]

The samples necessary for the laboratory assessments may be feasibly obtained from infants, including cord blood, small samples of peripheral blood mononuclear cells from venous samples which potentially could be obtained from left over specimens acquired for other purposes, or possibly from capillary samples. Since HHV6 is persistent in the saliva, such respiratory samples may be easily obtained without discomfort to the infant, and urine samples may be obtained by extracting urine from diapers. Assessment of hearing is recommended by the American Academy of Pediatrics[4] during the newborn period and subsequent evaluations along with the neurodevelopmental assessments are non-invasive and generally acceptable by families.

References:
1. Caserta M, Hall C, Schnabel K, McIntyre K, Long C, Costanzo M, Dewhurst S, Insel R, Epstein L. Neuroinvasion and persistence of human herpesvirus-6 in children. J Infect Dis 1994; 170:1586-1589.
2. Hall C, Long C, Schnabel K, Caserta M, McIntyre K, Costanzo M, Knott A, Dewhurst S, Insel R, Epstein L. Human herpesvirus-6 infection in children: a prospective study of complications and reactivation. N Engl J Med 1994; 331:432-438.
3. Carney A, Moeller M. Treatment efficacy: Hearing loss in children. J Speech & Hearing Res 1997; 41:S61-S84.
4. American Academy of Pediatrics A. Task Force on Newborn and Infant Hearing - Newborn and Infant Hearing Loss: Detection and Intervention. Pediatrics 1999; 103:527-530.
5. Yoshinaga-Itano C, Sedey A, Coulter D, Mehl A. Language of early- and later-identified children with hearing loss. Pediatrics 1998; 102:1161-1171.
6. Harrison M, Roush J. Age of suspicion, identification, and intervention for infants and young children with hearing loss: A national study. Ear & Hearing 1996; 17:55-62.
7. Stein L, Jabley T, Spitz R, Stoakley D, McGee T. The hearing-impaired infant: patterns of identification and habiliation revisited. Ear & Hearing 1990; 11:201-205.

8. Dalzell L, Orlando M, MacDonald M, Berg A, Bradley M, Cacace A, Campbell D, DeCristofaro J, Gravel J, Greenberg E, Gross S, Pinheiro J, Regan J, Spivak L, Stevens F, Prieve B. The New York State Universal Newborn Hearing Screening Demonstration: ages of hearing loss identification, hearing aid fitting, and enrollment in early intervention. Ear Hear 2000; 21:118-130.
9. Levy S, Hyman S. Pediatric assessment of the child with developmental delay. Pediatr Clin North Am 1993; 40:465-477.
10. Adams O, Krempe C, Kogler G, Wernet P, Scheid A. Congenital infections with human herpesvirus 6. J Infect Dis 1998; 178:544-546.
11. Dahl H, Fjaertoft G, Norsted T, Wang F, Mousavi-Jazi M, Linde A. Reactivation of human herpesvirus 6 during pregnancy. J Infect Dis 1999; 180:2035-2038.
12. Daibata M, Kamioka M, Wakiguchi H, Miyoshi I, Taguchi H. Human herpesvirus 7 and lymphocytic leukaemia. Br Haemotol 1999; 104:934.
13. Maeda T, Okuno T, Hayashi K, Nagata M, Ueda M, Terashima K, Kawashima T, Miyamoto H, Mori T, Yamada Y. Outcomes of infants whose mothers are positive for human herpesvirus-6 DNA within the genital tract in early gestation. Acta Paediatr Japonica 1997; 39:653-657.
14. DesJardin J, Gibbons L, Cho E, Supran S, Falagas M, Werner B, Snydman D. HHV6 reactivation is associated with cytomegalovirus infection and syndromes in kidney transplant recipients at risk for primary cytomegalovirus infection. J Infect Dis 1998;178:1783-6.
15. Griffiths P, Ait-Khaled M, Bearcroft C, Clark D, Quaglia A, Davies S, Burroughs A, Rolles K, Kidd I, Knight S, Noibi S, Cope A, Phillips A, Emery V. Human herpesvirus 6 and 7 as potential pathogens after liver transplant: prospective comparison with the effect of cytomegalovirus. J Med Virol 1999; 59:496-501.
16. Miyoshi H, Tanaka-Taya K, Hara J, Fujisaki H, Matsuda Y, Ohta H, Osugi Y, Okada S, Yamanishi K. Inverse relationship between human herpesvirus-6 and -7 detection after allogenic and autologous stem cell transplantation. Bone Marrow Transplant 2001; 27:1065-1070.
17. Alford C, Stagno S, Pass R, Britt W. Congenital and perinatal cytomegalovirus infections. Rev Infect Dis 1990; 12:S745-S753.
18. Dahle A, Fowler K, Wright J, Boppana S, Britt W, Pass R. Longitudinal investigation of hearing disorders in children with congenital cytomegalovirus. J Am Acad Audiol 2000; 11:283-290.
19. Demmler G. Congenital cytomegalovirus infection. Semin Pediatr Neurol 1994; 1:36-42.
20. Fowler K, McCollister F, Dahle A, Boppana S, Britt W, Pass R. Progressive and fluctuating sensorineural hearing loss in children with asymptomatic congenital cytomegalovirus infection. J Pediatr 1997; 130:624-630.
21. Numazaki K, Chiba S. Current aspects of diagnosis and treatment of cytomegalovirus infections in infants. Clin Diagn Virol 1997; 8:169-181.
22. Csoma E, Bàcsi A, Liu X, Szabó J, Ebbesen P, Beck Z, Kónya J, Andirkó I, ENagy E, Tóth F. HHV6 variant A infects human term syncytiotrophoblast in vitro and induces replication of human immunodeficiency virus type 1 in dually infected cells. J Med Virol 2002; 67:67-87.
23. Baillargeon J, Piper J, Leach C. Epidemiology of human herpesvirus 6 (HHV-6) infection in pregnant and nonpregnant women. J Clin Virol 2000; 16:149-157.
24. Chan P, Chan M, Li W, Chan D, Cheung J, Cheng A. Association of human beta-herpesviruses with the development of cervical cancer: bystanders or cofactors. J Clin Pathol 2001; 54:48-53.
25. Leach C, Newton E, McParlin S, Jenson H. Human herpesvirus 6 infection on the female genital tract. J Infect Dis 1994; 169:1281-1283.
26. Montgomery R, Youngblood L, Medearis D. Recovery of cytomegalovirus from the cervix in pregnancy. Pediatrics 1972; 49:524-531.
27. Okuno T, Oishi H, Hayashi K, Nonogaki M, Tanaka K, Yamanishi K. Human herpesvirus 6 and 7 in cervixes of pregnant women. J Clin Microbiol 1995; 33:1968-1970.

28. Romano N, Romano F, Viviano E, Vitale F, Villafrate M, Perna A, Bonura F, Guttadauro R. Rare association of human herpesvirus 6 DNA with human papillomavirus DNA in cervical smears of women with normal and abnormal cytologies. J Clin Microbiol 1996; 34:1589-1591.

29. Stagno S, Reynolds D, Tsiantos A, Fuccillo D, Long W, Alford C. Comparative serial virologic and serologic studies of symptomatic and subclinical congenitally and natally acquired cytomegalovirus infections. J Infec Dis 1975; 132:568-577.

30. Stagno S, Pass R, Dworsky M, Alford C. Maternal cytomegalovirus infection and perinatal transmission. Clin Obstet Gynecol 1982; 25:563-576.

31. Aubin J, Poirel L, Agut H, Huraux J, Bignozzi C, Brossard Y, Mulliez N, Roume J, Lecuru F, Taurelle R. Intrauterine transmission of human herpesvirus 6. Lancet 1992; 340:482-483.

32. Mendel I, De Matteis M, Bertin C, Delaporte B, Maguer D, Collandre H, Buffet-Janvresse. Fulminant hepatitis in neonates with human herpesvirus 6 infection. Pediatr Infect Dis J 1995; 14:993-997.

33. Wiersbitzky S, Ratzmann G-W, Bruns R, Wiersbitzky H. Reactivation in children of juvenile chronic arthritis and chronic iridocyclitis associated with human herpesvirus-6 infection. Padiatr Grenzgebiete 1993; 31:203-205.

34. Plotkin S. Vaccination against cytomegalovirus, the changeling demon. Pediatr Infect Dis J 1999; 18:313-325.

35. Boppana S, Pass R, Britt W. Virus-specific antibody responses in mothers and their newborn infants with asymptomatic congenital cytomegalovirus infections. J Infect Dis 1993; 167:72-77.

36. Boppana S, Britt W. Antiviral antibody responses and intrauterine transmission after primary maternal cytomegalovirus infection. J Infect Dis 1995; 171:1115-1121.

37. Boppana S, Miller J, Britt W. Transplacentally acquired antiviral antibodies and outcome in congenital human cytomegalovirus infection. Viral Immunol 1996; 9:211-218.

38. Harrison C, Britt W, Chapman N, Mullican J, Tracy S. Reduced congenital cytomegalovirus (CMV) infection after maternal immunization with a guinea pig CMV glycoprotein before gestational primary CMV infection in the guinea pig model. J Infect Dis 1995; 175:1212-1220.

39. Plotkin S, Smiley M, Friedman H, Starr S, Fleisher G, Wlodaver C, Dafoe D, Friedman A, Grossman R, Barker C. Towne-vaccine-induced prevention of cytomegalovirus disease after renal transplants. Lancet 1984; 1(8376):528-30.

40. Schoppel K, Schmidt C, Einsele H, Hebart H, Mach M. Kinetics of the antibody response against human cytomegalovirus-specific proteins in allogeneic bone marrow transplant recipients. J Infect Dis 1998; 17:1233-1243.

41. Dockrell D, Paya C. Human herpesvirus-6 and -7 in transplantation. Rev Med Virol 2001; 11:23-36.

42. Norton R, Caserta M, Hall C, Schnabel K, Hocknell P, Dewhurst S. Detection of human herpesvirus 6 by reverse transcription-PCR. J Clin Microbiol 1999; 37:3672-3675.

43. Kondo K, Shimada K, Sashihara J, Tanaka-Taya K, Yamanishi K. Identification of human herpesvirus 6 latency-associated transcripts. J Virol 2002; 76:4145-4151.

44. Harrison W, Dunnell J, Mascher K, Fletcher K, Vohr B, Gorga M, Widen J, Cone-Wesson B, Folsom R, Sininger Y, Norton S. Identification of neonatal hearing impairment: experimental protocol and database management. Ear Hear 2000; 21:357-372.

45. Canfield R, Smith E, Brezsnyak M, Snow K. Infant information processing through the first year of life: a longitudinal study using the visual expectation paradigm. Monogr Soc Res Child Dev 1997; 63: Whole Issue.

46. Davidson P, Weiss B, Myers G, Cory-Slechta D, Brockel B, Young E, Orlando M, Loiselle D, Palumbo D, Pittelli R, Sloan-Reeves J. Evaluation of techniques for assessing neurobehavioral development in children. NeuroToxicology 2000; 21:957-972.